Studies for the ultrasound application in winemaking for a low impact enology

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Abstract. In winemaking, although some research has been carried out, the practical application is still in an early stage. The cavitation process induced by ultrasounds leads to the breakage of the cells walls, thus facilitates the release of the cell content in the wine. The aim of this work is to study the effects produced by the ultrasound on crushed grapes, seeds, lees, and wines, in order to verify the possible speeding up of the extraction, the polymerization of the phenolic compounds, and the release of compounds from the yeast cells. After some preliminary tests, an experimental design has been created to optimize the test conditions, like time, amplitude and frequency. After different laboratory activities, recent experience in the cellar with a prototype have confirmed the possibility of using ultrasound for a short time (3-5 minutes) as a pre-treatment on crushed red grapes for the extraction of polyphenols and on crushed white grapes for the extraction of aromas. The treatment on the lees has confirmed the possibility of shortening the time of aging on the lees. Also good results have been reached in young red wines using the ultrasound to promote a positive tannin evolution. Experiences are ongoing to create an industrial plant for treatment with ultrasound in cellar, with variable flow rates between 50 and 300 hL/h; we are also being studied further applications in other stages of the winemaking process.

Introduction

The ultrasound technology is based on mechanical waves at a frequency higher than the upper limit of human hearing (>16 kHz). Being sound waves, ultrasound is transmitted through any substance, solid, liquid or gas, which possesses elastic properties. These waves travel either through the bulk of a material or on its surface at a speed which is characteristic of the nature of the wave and the material through which it is propagating [1, 2, 3]. In the food industry, ultrasound can be classified into two frequency ranges: high frequency ultrasound (100 kHz – 1 MHz) and power ultrasound (16 – 100 kHz).

Power ultrasound has been used for many years to generate emulsions, disrupt cells, and disperse aggregated materials; more recently, some applications have been identified with greater potential for future development, i.e., modification and control of crystallization processes, degassing of liquid foods, enzymes inactivation, enhanced drying and filtration and the induction of oxidation reactions [3, 4, 5].

The properties of power ultrasound waves have generated interest in the food industry, since the promotion of physical and chemical reactions can lead to a strategic advantage in processing. Since the '90s, ultrasound treatments have become a widely applicable alternative in the processing industry [6]; ultrasound is applied to food technology for its mechanical and/or chemical effects on homogenization, mixing, extraction, filtration, crystallization, dehydration, fermentation, degassing, defoaming, particle size reduction, temporary or permanent changes of viscosity, modulation of growth of living cells, cells disruption and aggregate materials dispersion, inactivation of microorganisms and enzymes, sterilization of equipment [7, 8, 9]. For these reasons, the effects of this technology are interesting in the food industry, but there has not been much research done on fermented beverages, especially regarding the winemaking process.

Guerrero et al., [10] have published a study on the ultrasound effects on Saccharomyces cerevisiae survival after application of wave amplitude, in the range of 71 to 110 µm and a frequency of 20 kHz. The microscopic observations determined that ultrasound caused puncturing of cell walls with leakage of content as well as damage at subcellular level.

Ultrasound has recently been applied to evaluate the possible effect on color and flavor extraction at different stages of the winemaking process [6, 11, 12, 13]: red color and aromatic compounds are localized in the skin cells and their release is facilitated by mechanical actions, disruption of tissues and cells, temperature and alcohol.

In addition to this, in the last few years, some researchers have focused their attention on wine aging accelerated by the application of physical methods, unlike chemicals, such as using ultrasound [14, 15, 16, 17, 18, 19].

Red grapes and their phenolic compounds are the most important raw material for winemaking. Anthocyanins and tannins are the main phenolic compounds responsible for color, taste, and aging properties of the red wine. Both compounds are found in berry skins, in the cell vacuoles, although tannins are also found in the seeds. The extraction of skin phenolic compounds during fermentative maceration is essentially a diffusion process, and the rate of extraction is influenced by the skin phenolic concentration, the composition of berry cell walls, and the technological process applied to the winery [20].

Innovative technologies, including ultrasound, have been recently tested to improve the extraction of phenolic compounds from grape berries, grape mash, grape skin, and seeds [11, 8, 12, 13]. Ultrasound can effectively enhance the extraction of intracellular compound from different plants. El Darra et al. [12] summarizes what literature has reported: the ultrasound application at 20-35 kHz enhances the extraction of polyphenols from red grape residues and from grape seeds of phenolic and other bioactive compounds from grape must and the flavonoid extraction from grape skin without the undesirable degradation of phenolics.

Ultrasound could also promote all the reactions which occur during the aging process, such as oxidative reactions (involved or not molecular oxygen) and reductive reactions.

One of the most important changes during aging is a progressive increase and stabilization of the color due to copigment anthocyanin complexes, the formation of new pigments and the progressive formation of both tannin-tannin and anthocyanin-tannin complexes [21, 22].

Several studies have literally illustrated the potential of alcoholic beverages aging by chemical methods [22, 23], while very few experiments have been done applying physical methods, such as ultrasound [14]. Ultrasound effects on red wine physicochemical properties have been recently investigated [17]. The authors have reported that different conditions of ultrasonic treatment have significantly changed the concentration of total phenolic compounds and the electrical conductivity. Therefore, this physical technology, modifying the characteristics of red wine, could be a promising method for accelerating the wine aging process [16].

Ultrasound could also be used to accelerate the release of protective colloids from lees and stabilize wines in a very short time [18, 19]. The ultrasound cavitation would be able to facilitate the release of these compounds, causing disruption of cell walls and membranes.

Ferraretto and Celotti [24] have recently explored the stabilizing effects of ultrasound on tannin-anthocyanins polymers, in order to understand how to speed up the aging reactions and hence reduce the time between production and consumption.

Ultrasound, as a relatively low-cost, non-hazardous and environmental friendly technology, is commonly used in the food industry [7, 25, 26] hence its possible application in the wine industry might become an important technological innovation by speeding up some slow reactions required in the winemaking process.

We think that a possible application of ultrasound in the wine industry could be an important technological innovation that would fasten the thermodynamically possible reactions, which are required in the winemaking process, although they are kinetically slow.

In this paper we present our recent studies for the application of ultrasonic technology in the winemaking processes.

Methods

Ultrasonic Equipment

An ultrasonic processor (SONOPLUS HD 2200, Bandelin electronic, Berlin, Germany) with 13 mm sonotrode probe, made of titanium, was used for sonication.

Samples were processed in a continuous sonication at a constant frequency of 20 kHz. The energy input was controlled by setting the amplitude of the sonicator probe; the total nominal output was 200 W. The ultrasound probe was submerged to a depth of 50 or 20 mm in the sample on the basis of the experiments.

Its basic components are the power generator and the transducer that converts electrical power into mechanical vibrations. A schematic model is shown in Figure 1.

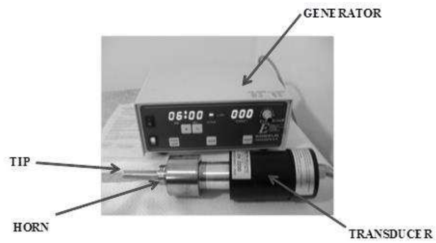

Figure 1. Ultrasound instrument for laboratory applications

The amplitude levels and the processing times were varied on the basis of the experiments and on the tests carried out prior to this study. The final treatment temperature was measured for all samples.

For the winery application we used a prototype at a frequency of 27 kHz with possibility to work in static and dynamic conditions; figure 2 shows the prototype used.

Figure 2. Prototype for cellar applications

Skin maceration trials

Phenolics Extraction from the Red Grape Skin

Samples: a wide set (nr. 40) of different varieties of red grapes sampled at technological maturity was evaluated for the tests conducted in laboratory. They were sampled directly upon delivery in the cellar, in order to have a representative and real sampling in relation to the ripening degree of the grapes delivered to the cellar.

A representative sample (250 g), a stalk removed and manually crushed, was subjected to the treatment in a beaker of 250 mL. The polyphenols extracted were evaluated in the liquid obtained by centrifugation from the treated grapes.

Treatment conditions: the conditions promising the best results in terms of extraction following a preliminary test (treatment time from 1 to 5 minutes at three different levels of amplitude – 30, 60 and 90%) were chosen for the laboratory tests.

To evaluate the immediate effect on the color, the treatment conditions were 2 minutes at different amplitudes (20, 40, 60 and 80%).

Micro-Vinification Trials

Samples: 800 g of crushed samples of the same grapevine variety (Merlot), but with a different polyphenolic content were used to perform maceration and fermentation trial in the cellar. The crushed grapes were previously subjected to ultrasonic treatment and then micro-vinified and racked sequentially. A reference sample, not treated with ultrasound, was analyzed for all the tests in order to have the sample that simulated the traditional maceration. All the samples, including the reference, were placed in open containers (2 L) and placed in the same conditions of maceration-fermentation in order to make comparable the phenolics extraction.

The effect on wine color was studied on micro-vinification trials of the samples performed directly in a cellar. The maceration time for the ultrasound treated samples was 2, 3 and 4 days, while for the control sample (untreated) it was 5 days, a treatment of 5 minutes at the amplitude level of 90% was carried out to assess ultrasound effects on the maceration kinetics.

Prototype experiences

During the 2014 and 2015 harvests we were made experiences of the cellar to check the effects of treatment with ultrasound on crushed red and white grapes. The tests were carried out at 27 kHz frequency of crushed grapes in the winery conditions. The goal was to reduce the maceration time for the red grapes, while in white grapes the objective was to eliminate maceration and optimize the extraction of aromas and aroma-precursors.

All the analysis was performed in triplicate. For the red grapes polyphenols were analyzed, while for white grapes also the aromatic substances were evaluated

Red Wine aging

Experimental tests, carried out to assess the possible effects of ultrasound on red wines, have been set according to different work plans.

For preliminary tests, all treatment combinations (time and amplitude %) were analyzed with the response surface method (RSM: Response Surface Methodology) in a model of experimental design compound central face-centered (CCF: Central Composite Face-Centered) in order to optimize the number of experiments and operating conditions, and to obtain the greatest number of data with the minimum number of samples.

The level of amplitude varied between 30% and 90% and the time of treatment between 1 and 5 minutes; the reference sample, not subject to treatment, was evaluated separately. The conditions for sample 3 were 1, 3 and 5 min at 30%, 60% and 90% (51, 102, 153 µm) of amplitude and each treatment was replicated 3 times.

The tests have been performed on different young red wines (4 months after racking) immediately after the treatment: 200 mL of wine for every sample have been treated.

Experiments were also carried out in cellar with the prototype on young red wines.

Effects of ultrasound treatment were evaluated using the Glories' index (Glories, 1978) which show the status of phenolic compounds in red wine, mainly based on the absorbance measured using a spectrophotometer.

Lees treatment

Treatment conditions: conditions that promised the best results in terms of extraction, following a preliminary test (treatment time from 1 to 5 minutes at three different levels of amplitude – 30%, 60% and 90%), were chosen for laboratory tests.

The fresh lees used were collected from different wineries after alcoholic fermentation. It was made the comparison with enzyme-treated lees and natural autolysis.

Analytical tests performed: Surface electrical charge of colloids, Turbidity, Protein stability tests Particle Size distribution, Soluble colloids by alcoholic precipitation, Protein quantification, Molecular mass determination by gel permeation high pressure liquid chromatography.

Also winery experiences have been made with the prototype in order to verify the real conditions of the treatment of yeast lees after alcoholic fermentation.

Statistical analysis

Analysis of Variance for one or more factors was applied on the collected data, using the Statistica for Windows v.7.0 software. Significance differences between trials were analyzed by the Tukey HSD test and are expressed with different letters. The experimental design was elaborated using Modde 8.0.2 Umetrics software.

Results

Extraction by Ultrasound on red and white grapes

As described in our previous work [13], we found that the 20 kHz ultrasound treatment in a solid-liquid medium, such as pressed crushed grapes, was responsible for accelerating the release and the dissolution of phenolic compounds contained in epidermal tissues.

The total polyphenol index, the anthocyanins content and the color intensity, related to the control sample extracted in a wine-like solution and in acidified methanol, proportionally increased (50% or more) with the time and amplitude of treatment for all grapevine varieties tested (analyzed). Under the same treatment conditions, the total polyphenol index and the anthocyanins content proved different depending probably on the characteristics (cell wall maturity, pectin content) of the different grapes varieties [13].

The tests carried out to evaluate the immediate effect on the color showed an important interaction between the cavitation phenomena and skin cell wall, leading to a higher color intensity and anthocyanins content. The compound extraction increases proportionally with the amplitude level (r2 = 0.9147) (Figure 3).

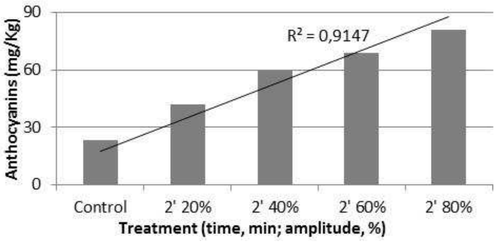

Figure 3. Anthocyanins extraction after Ultrasound treatment

Also the micro-vinification of samples treated with ultrasound provided interesting results. The crushed treated vinified samples had higher polyphenol content in comparison with the reference at the beginning of maceration. The same was found at the end of the process and it influenced the total polyphenol index more than the anthocyanins content.

The different series gave different results, especially samples with a higher initial phenolic content. Generally, it is possible to affirm that the variability of the ultrasound treatment effect depends on the quality of the raw material.

Focusing on process optimization, a maximum of three days maceration time could be saved with respect to the five days in the classical vinification. The results have shown a reduction from 1 to 3 days of maceration time for both the phenolic and anthocyanins content. Consequently, the ultrasound treatment could be considered an auxiliary technology during maceration.

Experiences of white grapes have confirmed the applicability of ultrasonic treatment to eliminate the traditional maceration and optimize the extraction of aromas and aromas precursors without exceeding the extraction of instable polyphenols [27].

In the figure 4 are shown some positive results obtained with the prototype in the 2015 harvest on Traminer. For all the analyzed aroma compounds there are increments related to the effect of ultrasonic treatment.

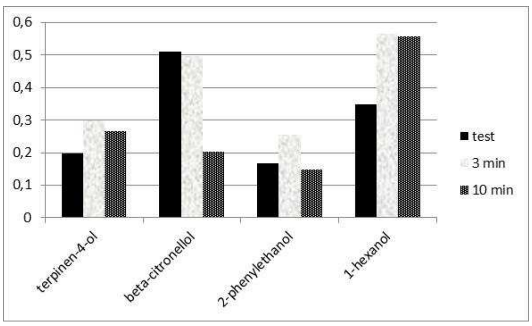

Figure 4. Effect of Ultrasound on bound-aroma compounds on Traminer grape

Other experiences on Glera (Prosecco) and Moscato grapes have demonstrated the applicability of continuous treatment with Ultrasound on crushed grapes to eliminate the traditional maceration by passing the crushed grapes on ultrasound reactor for few minutes and then directly in pressing.

Effects on Young Red Wine

In our latest study [24], we found that the addition of mechanical and chemical energy to the young red wine causes some modifications: by comparing different samples, we noticed that the ultrasound applied to wines with different composition leads to different results.

Since the temperature rises during the treatment, resulting into possible changes on the treatment effects and final wine quality, this parameter was monitored and recorded during the treatments. The increase in temperature from the standard 20ºC is related to treatment times and power. The temperature increased to a maximum of 43.5ºC.

This increase of temperature is not to be considered a problem in a potential future industrial application; the increase which could occur in few minutes of treatments does not cause a loss of wine color quality, as found by Galvin’s studies. Besides, it has to be considered that it is possible to carry out the treatment in controlled temperature conditions, if necessary, to avoid undesirable increases of wine temperature.

It is interesting to notice that no negative consequences on the anthocyanins, either loss of color, appeared after the treatment. Free anthocyanins were not modified, thus confirming their chemical stability under the treatment conditions applied. The stability of free anthocyanins is very important because they can combine later with tannins to obtain stable macromolecular complexes.

The most interesting results were achieved with tannic compounds. The figure 5 shows interesting results on tannin polymerization (HCl index) using different time and amplitude conditions.

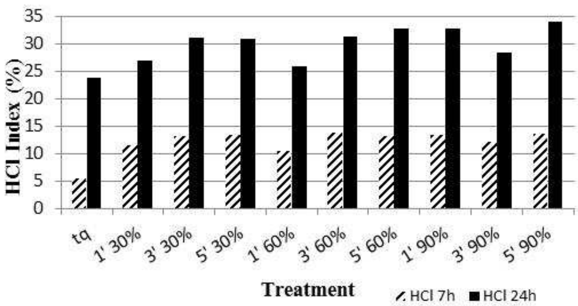

Figure 5. Effect of Ultrasound on tannin polimerization of young red wine.

The catechin content analysis showed an increase in values on all samples; the treatment probably promoted the liberation of the monomeric catechins from tannins with different effects on their reactivity (tannins reactivity). Statistical parameters of the response surface model present a good correlation coefficient for catechins (r2 = 0.8989, r2 Adj. = 0.7977). A significant increase of catechins was observed only for high values of amplitude and longer times of treatment. In the others conditions the results highlight the interaction between variables and accordingly the need of ultrasound treatments with specific combinations of amplitude and time based on the result expected. We could suppose different treatment conditions depending on the ratio between the

polyphenols and mainly in function of the tannic structure of the wine, closely related to the content of catechins.
According to these results, we affirm that a few minutes treatment could be a valid approach to manage some parameters in the aging of red wines; in particular, the increase of HCl index related to tannins polymerization contributes to the wine astringency reduction.
Considering the ultrasound treatment has a different effect on different wines, this approach could be useful, if confirmed by other experiments, to manage the tannin polymerization level to be able to control the chemical and physical stability of the tannic macromolecules and their astringency. The index of ethanol also led to significant results, as its changes might have supported a rearrangement, probably due to an increasing reactivity of the polysaccharides and tannin compounds.
The figure 6 shows, after 45 days from treatment, positive results on polymerization complexes Tannin-Anthocyanin, it confirm the possibility to use the ultrasound to promote some polymerization and improve the color stability [28].

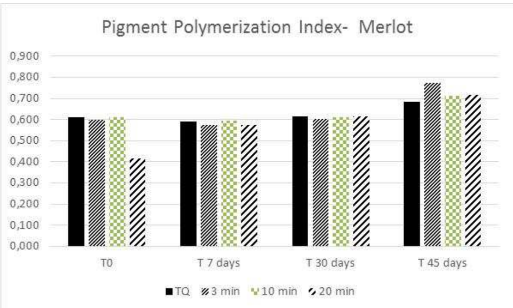

Figure 6. Effect of Ultrasound on color stability of young red wine.

The astringency, evaluated by gelatin index, increases with short and mild treatment, while is reduced producing a pleasant sensation with stronger treatment conditions, particularly at the conditions of 5 minutes at 60% of amplitude and 1, 3 and 5 minutes at 90% of amplitude.. The different modifications of the gelatin index, directly related to astringency, might confirm the hypothesis that the ultrasound treatment conditions can be modulated depending on the tannic composition of the wine, making it possible to manage astringency perception in a short time.

Effects on Yeast Lysis

Ultrasound causes cell disruption and this leads to the precipitation of certain colloids (polysaccharides and glycoproteins) or their separation by centrifugation. In this experiment it was possible to notice that the enrichment of the medium increased after the treatment with a higher impact of time rather than percentage of amplitude. The values of the sample treated for 5 minutes are tripled compared to the untreated sample. This remarkable increase is significant also from the technological point of view in the case of applying the ultrasonic treatment in wines fining on lees.
To understand which substances were influenced by the treatment, an evaluation of the proteins present in the soluble colloids and a protein test stability were performed.
The protein content measured in the lees supernatant gave values greater than the total colloids. The increase in proteins confirms the disruptive effect of ultrasound on yeast cellular structures, so it is probably possible to assume significant positive or negative effects on other cellular components.
Taking into account all the analyses performed for the experimental studies, the ultrasound treatments giving major effects were the ones at 5 minutes and 60% amplitude and those at 3 and 5 minutes at 90% amplitude (the maximum temperature reached by the sample with the strongest treatment was 47.6 °C; this temperature could probably not be of concern for the quality of the final product, considered the short time of treatment and the fact that only the liquid lees are treated, and not all the wine to be processed).
In the figure 7 the results on total soluble colloids are reported. For short treatment time there is a significant increase of extracted soluble colloids.

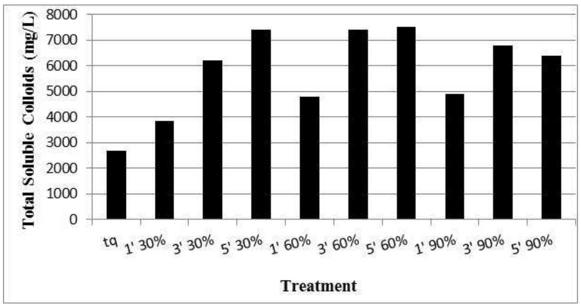

Figure 7. Increase of total soluble colloids in lees after ultrasound treatment

Lower treatment times, 0-10-20 days (lower increase in temperature of the sample), were chosen for the comparative study.
The same wine lees used for experimental studies were used in this long term trial. Three 750 mL bottles per treatment and kept at the cellar temperature for over seven months: ultrasound treatment (3 minutes at 90 % amplitude), β-glucanase treatment (40 mg/L) and natural autolysis control. The analyses were performed at time zero, after ten days, after one month, after three months and after seven months. Considering the total colloids over time they were double three months after the treatment and this value was double at seven months after the treatment. Total colloid increased for all treatments but the highest results were obtained with the enzyme-treated lees. Looking at the immediate effects, the analysis performed at time zero right after the ultrasound treatment showed a big increase compared to the control. Colloids released after 30 days with no treatment were equivalent to those obtained in only 10 days with ultrasound assisted lysis. The analyses performed at 1, 3 and 7 months time showed that colloids released naturally by lees autolysis were equal to those treated with ultrasound; therefore the interest remains only on the immediate effects.
Indeed, this research aims to identify the operating conditions of treatment of a lees sample in order to

reduce the traditional natural lysis time (months) and/or eliminate the addition of exogenous β-glucanase.

HPLC-gel permeation analysis with two types of detectors allows to separate different fractions of polysaccharides and proteins. It showed the same fractions among the different treatments for both detectors. Interestingly, one fraction (86.24 kDa) presented differences due to the treatment: it was higher in ultrasound treated lees compared to the control both at time 0 and 10 days, but lower than in the enzyme-treated lees at 10 days. This can be explained by considering that in 10 days under those conditions, the activity of the added enzyme made a significant impact. The results confirm the importance and usefulness of the Ultrasound technology in the wine industry for the yeast lysis in a short time. Differences among treatments appeared clearer in the short-term, but did not show in the long-term period. Considering that the interest of this technology is to accelerate the positive effects of ageing over lees, reducing the time needed with usual practices, it was decided to make experiences with the prototype in cellar conditions.

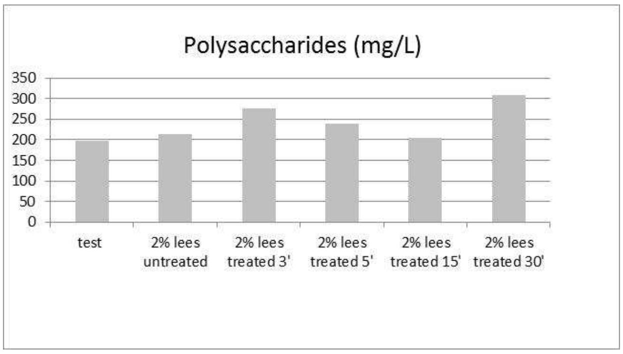

Figure 8. Polysaccharides after addition of different percentages of lees treated with Ultrasound

The figure 8 represents the results of white wine Crossing Manzoni 6.0.13 microfiltered, added to different percentages of lees treated with ultrasound with the prototype in cellar conditions. The data confirm the interest of the lees treatment to be able to add to the young wine a high content of soluble colloids, without long periods of aging on lees.

Ultrasound treatment on wine lees had a bigger impact in terms of the treatment time than its intensity, in the range of trials essayed. The ultrasound treatments giving major effects were the ones at 5 minutes and 60% amplitude and those at 3 and 5 minutes at 90% amplitude.

From this research the parameters that increased correlated with time and intensity of treatment were total colloids and proteins, which was a consequence of an increased release of colloids from the yeast. A fraction characterized by HPLC analysis by both UV and RI detector and suspected to be of a glycoprotein nature, increased with the treatment intensity.

The total colloids and negative charge increase in the US-treated samples was statistically different from the control and the enzyme treatment only immediately after the treatment and after 10 days; therefore we can conclude that the advantage of this new technological application can be a time reduction of the ageing process.

The results show the significant effect of the ultrasound treatment on obtaining a rapid extraction of macromolecules from the yeast lees, useful for the colloidal and aromatic evolution of a wine. The result is even more significant if it is considered that within a few minutes of the Ultrasound treatment, comparable effects to traditional ageing on lees with or without added enzymes are obtained..

Conclusions

The application of ultrasound in winemaking represents, first of all, a possibility to optimize fermentation technology and, therefore, a better management of red grape vinification. Furthermore, the application of ultrasound can be considered as a continuous pre-treatment of crushed red grapes before loading the vinification tank, in view of the very short treatment times. The tests carried out on different grape varieties showed indeed how a few minutes of treatment at different amplitude levels can achieve an enhancement in the extraction of phenolic compounds, and in terms of time, a progressive reduction in the time of the classic maceration. The treatment of the crushed white grapes instead eliminates the traditional maceration and ensure a balanced extraction of aromas without exceeding the extraction of oxidizable polyphenols.

From the results obtained on wines after treatment with ultrasound, some indications suggest the use of the ultrasound to promote the polymerization of the phenolic compounds as the wine matures and therefore accelerate the aging process of wines. This technology can give the best result in the treatment of young, well colored wines featured by an immature tannic structure which is still evolving.

A brief ultrasound treatment could replace or integrate the traditional aging and stabilization techniques that are often constrained by the length of the kinetics of reactions between polyphenolic molecules.

It is also necessary to further investigate on the kinetics and response times to optimize the ultrasound technology on the treatment of red wines.

The treatment of white fermentation lees after fermentation promotes the lysis of yeast with a rapid release of colloids, polysaccharides, and mannoproteins, and stabilize the wine. These effects can provide also a possible reduction in wine ageing on lees.

Recent experience during the 2016 harvest have also highlighted the possibility of quick continuous treatment of the flotation lees to improve the filterability and thus the efficiency of the cross flow filtration of the lees. Other experiences have also allowed us to improve the filterability of wine lees.

All technological experiences were also evaluated using sensory analysis of the wines, in all cases there was a positive response of the ultrasound on the sensory characteristics of the wines.

The results obtained confirm the extractive and lytic effects of the ultrasonic treatment on food matrices and could be applied certainly on a technological level to

optimize some technological processes in the wine industry.
Other experiments are in progress to evaluate further applications of ultrasonic technique in the winemaking processes. Ultrasound can be considered a new and sustainable technology for low impact winemaking: this technique could reduce the use of sulfur dioxide and enzyme for the extraction of phenolic compounds and, stabilize the wine without the use of exogenous adjuvants from yeast.
A winemaking with ultrasounds could result favorable to optimize costs of process, reduce time, and re-utilize by-products of vinification respecting the quality of the raw material.

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